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(54) Title: METHOD AND APPARATUS FOR TRANSFERRING SMALL VOLUME LIQUID SAMPLES

(57) Abstract: Microtiter-like plates are described which have paired wells on opposite sides of the plate that are connected so that liquid can flow freely between the wells in each pair. One well (the "sampling port") in each pair is a "virtual" well having a very small volumetric capacity compared with the other well in the pair, which also may be a virtual well. Virtual wells confine small volume liquid samples on a relatively flat surface by surface tension. Liquid samples partition between the larger well and the sampling port so that a relatively constant volume of fluid remains in the sampling port even as the liquid volume in the larger well changes. The consistent liquid volume in the sampling port enables reproducible liquid volumes to be transferred from the sampling

TITLE OF THE INVENTION

METHOD AND APPARATUS FOR TRANSFERRING SMALL VOLUME LIQUID SAMPLES

FIELD OF THE INVENTION

The present application relates to microtiter-like plates and transfer devices having arrays of pins that may be used for handling and transferring small volume samples and arrays of samples of liquids for such purposes as screening of biological materials and biologically active compounds.

BACKGROUND OF THE INVENTION

Current methods of drug discovery often involve assessing the biological activity (*i.e.*, screening) of tens or hundreds of thousands of compounds in order to identify a small number of those compounds having a desired activity. The assays are generally carried out in multi-well tissue culture plates called microtiter plates. Microtiter plates are usually made of plastic, with the wells being formed by indentations in the bottom of the microtiter plate. For screening, commonly used microtiter plates have 96 individual wells, although the trend is to use higher density plates of 384, 864, 1536, 3456, and even 9600 wells. Currently 96 well plates are made in a broad variety of shapes, colors, materials, and sizes, but they all have wells that hold volumes of at least tens of microliters, require individual dispensing of reagents into each well, and require individual washing of each well except in the case of selected assays in filter bottom plates. Higher density plates typically have wells that hold lower volumes, but such plates are subject to more limitations in that few such plates are available with filters in the bottom and assay performance is often compromised. For many purposes, plates with more than 384 wells are not practical. For example, the transfer of fluids into and out of the narrow wells of such plates is very difficult and requires very precise pipetting.

In general, it is desirable to utilize microtiter plates having the largest possible number of wells per plate and the smallest possible volume per well in order to maximize the throughput and minimize the mechanical complexity of high throughput screening operations. In addition, the use of smaller volumes per assay is desirable for a number of reasons: conservation of scarce biological and chemical materials, more efficient use of reagents, ability to run assays on primary cells, ability

to develop assays faster due to reduced reagent purification, fewer plates needed to run a given number of assays and thus fewer handling problems and less storage space needed.

While it is desirable to decrease the size of wells in current microtiter plates, there are problems associated with doing so, including for example difficulty in pipetting fluids into confined spaces, inadequate and slow mixing, difficulty effecting separations, rapid evaporation times, and limited signal strength during measurement. Copending and commonly assigned PCT Application No. US 99/02300, filed February 3, 1999, addresses these problems, and thereby provides microtiter plates containing a larger number of wells that hold on the order of 10 nl to 10 µl of liquids; that are easy to pipette into; that facilitate fluid transfer; that minimize mixing time; that allow for easy separations and washing. The same patent application also discloses methods for minimizing evaporation from the small volume liquid samples.

The problem of dispensing and transferring small volumes of liquid samples and arrays of liquid samples continues to be a major obstacle toward further miniaturization. In the past, the problem of dispensing small volumes containing compounds of interest into or out of the wells of microtiter plates has been accomplished by use of metal pins that need to be washed after each use (such as on the BioMek 2000 High Density Replicator (HDR) tool, see, *e.g.*, Brandt, 1997, J. Biomolec. Screen. 2:111-116); or by pin replicators (such as the pin replicator made by V&P Scientific, Inc., 1997, J. Biomolec. Screen., 2: 118). The prior art pin tools or pin replicators have disadvantages in that they need to be washed, leading to possible contamination and loss of time, require relatively large volumes, do not have the accuracy needed, or are too expensive. Furthermore, they are less effective as fluid volumes get smaller. Finally, the pins are long and thin, so that using arrays of pins for plates having more than 384 wells is very difficult because the pins cannot be made straight enough for extended periods of time.

Alternatively, samples may be manipulated by aspirating a relatively large volume (usually at least 100 nl and generally at least a few µl of solution) with a low volume pipetter such as the Packard piezoelectric pipetter or Cartesian's solenoid based pipetter. Pipetters, such as those listed above, are very slow. Also, like the prior art pins, they need to be washed, leading to possible contamination and loss of

time. The pipetters also require significant dead volumes in the 10s if not 1,000s of nanoliters.

Given the difficulties involved in dispensing and removing reagents or compounds from the wells of microtiter plates, there is a clear need for better methods and better equipment for transferring and dispensing small volumes of reagents from multiple wells simultaneously, without the need to wash the device and pins between uses and without the need to use pipetters or pins that hold liquids in grooves by capillary action. The apparatus and methods described below significantly simplifies these transfers of liquids

## SUMMARY OF THE INVENTION

The microtiter-like storage plate disclosed herein is particularly useful for dispensing and and transferring uniform quantities of small-volume liquid samples, and especially arrays of liquid samples, quickly, and with minimal loss of material. The microtiter-like plate has a first side and a second side and comprises:

(a) an array of storage wells on the first side of the plate; and (b) an array of sampling ports on the second side of the plate,

wherein the storage wells on the first side of the plate and the sampling ports on the second side of the plate are paired, so that each storage well that is on the first side of the plate is paired with a sampling port that is directly opposite it on the second side of the plate to form a pair of one storage well and one sampling port, with each pair of one storage well and one sampling port being connected through the inside of the plate, so that liquid can flow between the paired storage well and sampling port, but not laterally in the plane of the plate to other wells or sampling ports;

wherein the sampling ports on the second side of the plate are "virtual wells, " where each virtual well has a hydrophilic domain surrounded by a hydrophobic field;

wherein the storage wells on the first side of the plate have the capacity to hold a larger volume of liquid than the sampling ports on the second side of the plate, and the sampling ports on the second side of the plate hold a volume of liquid that varies little with changes in the combined volume of liquid that may be in the paired storage wells and sampling ports on the two sides of the plate.

Because the storage wells and sampling ports are interconnected, liquid that is in the storage wells and sampling ports is redistributed as liquid is removed from or added to either of them. Because of the design of the wells and ports, most of the volume is retained in the storage well. The amount of liquid in the sampling port is relatively invariant regardless of the total volume This may be due to surface tension effects.

"Virtual wells" are disclosed in commonly assigned, copending PCT Application No. US 99/02300, filed February 23, 1999. They are also described in more detail in the Detailed Description section of this application.

A combined apparatus for transferring an array of liquid samples of approximately constant volume from the microtiter-like plate described above is also new. The combined apparatus comprises:

(1) the microtiter-like plate described above, and

(2) a transfer device that comprises an array of pins,

where the array of pins matches the array of sampling ports on the second side of the storage plate;

where each pin has a face at one end, and the faces are all in a single plane. The surface area of the face of the pin is believed to primarily determine the volume of liquid removed from the sampling port. The faces at one end of each pin preferably have the same surface characteristics and the same areas to ensure uniform drop size.

The array of pins and the array of sampling ports "match" if the pattern of the pins and the pattern of sampling ports are superimposable. If they "match," then they can be aligned so that each pin in the pin array touches each sampling port in the array of sampling ports. Note also that the area of the face of the pin governs the volume of liquid that is picked up by the pin. Although the correctness of theories on how an invention works do not reflect on whether or not the invention is patentable, the applicants do not wish to be bound by any theory. It nevertheless appears that when the pin gets close enough to the sampling port to touch the drop of liquid, the liquid forms a thin layer between the face of the pin and the sampling port due to attractive forces similar to capillary action. When the pin and plate are separated, the amount of liquid that remains on the face of the pin is determined by the area of the face that was coated with liquid. Since the whole face is coated immediately prior to separation, it is believed that the area of the face determines the

volume of liquid that remains on the pin after separation. Reproducible and consistent volumes of liquid can be transferred using this method. Although transfer from the sampling port to a pin array is the preferred embodiment, it should be noted that the relatively constant volume of the liquid in the sampling port will greatly reduce the variability between sequential dispenses of any pin transfer system or even a direct transfer to a virtual well plate from the sampling ports.

The apparatus described above can be used to transfer an array of liquid samples simultaneously (i. e. all the liquid samples at the same time in one group) from a microtiter-like plate to a receiving device. The method includes the steps of:

(1) providing the combined apparatus described above, with liquid samples placed in the storage wells to form an array of liquid samples;

(2) placing the transfer device in close proximity to the microtiter-like plate, so that the faces of the pins of the transfer device and the matching sampling ports of the microtiter-like plate are sufficiently close that liquid from the sampling ports is transferred to the faces or tips of the pins;

(3) separating the transfer device and the microtiter-like plate; and

(4) transferring the array of samples from the transfer device to a receiving device.

In the transfer step (2) above, the tips or faces of the pins essentially form a thin planar void between the plate and pins at the sample ports during the introduction and withdrawal of the pins from the sampling ports. The void fills by capillary action, thus wetting the entire face of the pin and improving the reproducibility of the transfer.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A illustrates a view of the first side of a microtiter-like storage plate of the invention. In these figures, the storage plate comprises an alumina plate with a fluorocarbon polymer coating to create the hydrophobic field. The continuous dark area (1) is the fluorocarbon polymer coating. The white area (2) represents an uncoated area that is hydrophilic (e.g. alumina, which may be functionalized with a hydrophilic coating or treatment) and thus is a virtual well,

which in this case is a storage well. The small dark circle (3) in the middle of the white circle is the opening of a hole that goes through the plate to the well on the other side of the plate.

Figure 1B illustrates a view of the other (second) side of the same plate. The sampling ports (4) are also virtual wells, having an uncoated hydrophilic domain surrounded by a hydrophobic field, and are smaller in area and volume than the virtual wells on the first side of the plate. The hole openings (3) are also shown in the center of the virtual wells.

Figure 2 illustrates a cross-sectional side view of a plate cut through a row of wells. Four different configurations of the storage wells on the first side of each plate are shown. The sampling ports on the second side of the plate (lower side of the drawing) are virtual wells comprising a hydrophilic well surrounded by a hydrophobic field, shown also from above in Figure 1B. Holes (14) passing from the well and through the plate are also illustrated.

A. Side view of the plate in Figure 1A, where the storage wells (2) are hydrophilic virtual wells surrounded by a hydrophobic fluorocarbon polymer coating (1), as was also shown from above in Figure 1A.

B. Cavities (5) are cut into the top of the plate for larger volumes.

C. Storage wells (2) (virtual wells) are on the upper side of the figure. The plate is constructed of a mesh (6) that allows fluid to flow vertically, but not laterally (horizontally). The mesh is hydrophilic.

D. A mesh plate as in C, but with deeper wells in the form of cavities (5) on the upper side of the figure.

Figure 3 illustrates the apparatus in the process of being used to make a transfer. Figures 3A, 3B and 3C, illustrate a side view of the microtiter-like plate (7) and a side view of the transfer device (8). Each storage well is a virtual well. Each well on the first side of the plate (upper side of the drawing) has a drop of liquid (9) in it. Each sampling port is also a virtual well. The sampling ports have smaller drops of liquid (10) that have passed through the hole from the storage well. The pins (12)

of the transfer device (8) are aligned so that contact can take place between the faces (11) of the pins and the droplets of liquid (10) in the sampling ports.

In Figure 3A, the plates are lined up for liquid transfer.

In Figure 3B, the transfer device has been brought close enough to the second side of the storage plate for the faces of the pins to touch the liquid droplets on the sampling ports, causing the liquid to fill in the narrow gap between the faces of the pins and the sampling ports.

In Figure 3C, the storage plate and the transfer plate have been separated again, with a drop of liquid (13) now present on the face of each pin.

## DETAILED DESCRIPTION OF THE INVENTION

In more specific embodiments of the microtiter-like storage plate as described above, the storage wells on the first side of the plate are selected from the group consisting of cavities and virtual wells, where virtual wells comprise a hydrophilic domain surrounded by a hydrophobic field.

"Cavities" is a term that refers to wells in conventional microtiter plates, where the liquid in the well is confined to the well by the walls of the cavity rather than by forces like surface tension (e.g. hydrophilicity and hydrophobicity). Microtiter-like storage plates in which the storage wells on the first side of the plate are cavities typically would contain a volume of about 0.001 ml to about 1 ml.

In one embodiment of a microtiter-like storage plate as described above, the storage wells on the first side of the plate are virtual wells, where each virtual well comprises a hydrophilic domain surrounded by a hydrophobic field, with the hydrophilic domain having an area of about 0.38 $mm^2$ to about 19 $mm^2$. The hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.002 $mm^2$ to about 6.75 $mm^2$, with the proviso that the areas of the hydrophilic domains of the sampling ports are smaller than the areas of the hydrophilic domains of the storage wells.

In another embodiment of a microtiter-like storage plate in which the storage wells on the first side of the plate are virtual wells, each virtual well

comprises a hydrophilic domain surrounded by a hydrophobic field, where the hydrophilic domain has an area of about 0.75 $mm^2$ to about 3.1 $mm^2$. The hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.03 $mm^2$ to about 0.75 $mm^2$, with the proviso that the areas of the hydrophilic domains of the sampling ports are smaller than the areas of the hydrophilic domains of the storage wells. In another embodiment of this last storage plate, the hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.2 $mm^2$ to about 0.5 $mm^2$.

In a more specific embodiment of the microtiter-like storage plates in which the storage wells are conventional wells (i.e. cavities), hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.002 $mm^2$ to about 6.75 $mm^2$. In another more specific embodiment, the hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.03 $mm^2$ to about 0.75 $mm^2$. In an even more specific embodiment of the microtiter-like storage plates in which the storage wells are conventional wells (i.e. cavities), the hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.2 $mm^2$ to about 0.5 $mm^2$.

As previously stated, storage wells on the first side and sampling ports on the second side of the plate are paired together, where the wells in each pair are connected so that liquid can flow between a paired well and sampling port but not to other wells or sampling ports. In one embodiment, the wells are connected by a channel. The channel may be approximately cylindrical in shape, or the channel may be flared at the openings on the first and/or second sides of the plate at the bottom of the storage well and sampling port. The channel generally may have a cross-sectional area in the range of about $1x10^{-6}$ to about 1.0 $mm^2$ at the narrowest part of the channel (measured perpendicular to the walls of the channel) between the storage well and the sampling port.

The connections between the wells in each pair can be made in other ways also. For example, each storage well on the first side and each sampling port on the second side of the plate that are paired together can be connected by a number of channels or perforations, rather than just a single channel. The pairs of wells can also be connected by a porous or woven support that is relatively hydrophilic (i.e. hydrophilic relative to the hydrophobic field surrounding the hydrophilic domains of

the virtual wells). Examples of porous or woven supports include woven or fritted versions of each of the following kinds of materials: ceramics, silicon, glass, metal, plastic, a plastic with a hydrophilic surface, sponge, and zeolites.

The hydrophilic domains of the virtual wells on the first side of the plate and of the sampling ports on the second side of the plate are each made of a hydrophilic material. Examples include plain glass, derivatized glass, silanized glass, glass with absorbed biopolymer or non-biopolymer, indium tin oxide, other metal oxides, gold or other metals, silicon, ceramic, hydrophilic plastics, and surface-modified polystyrene. There is no requirement that the storage wells and the sampling ports be made of the same hydrophilic material, but practically speaking, the plate will normally be made from a hydrophilic material, and the hydrophobic field is conveniently made by applying a hydrophobic coating to a hydrophilic substrate. As a result, the hydrophilic domains will often all be made of the same material.

In many cases, the hydrophilic domains do not need to be very hydrophilic, and in some instances may be considered hydrophobic. They just need to be more hydrophilic than the hydrophobic material that makes up the hydrophobic field, since the liquid samples are held in place by a combination of repulsive forces from outside the wells and attractive forces from the inside of the well and within the liquid itself. This also depends on the solvent. DMSO or a DMSO-water solution is a good solvent for many biological and pharmaceutical-screening type manipulations. These solvents are so hydrophilic that they are easy to contain if an extremely hydrophobic field is used, even if the hydrophilic domains are somewhat hydrophobic.

Examples of materials that can be used or have been used to create a hydrophobic field include polyfluorocarbons or polyfluorocarbon beads; TEFLON® or TEFLON® beads; perfluoropropene polymer; paraffin or other waxes or oils; polyethylene or other hydrocarbons; glass treated with chlorodimethyl octyl silane or other silanizing agents; polypropylene or other hydrophobic polymers; bicomponent materials containing beads or other hydrophobic insoluble materials, optionally including a binder; polyfluorocarbon beads or polyfluorocarbon-coated beads, optionally in a binder; and hydrocarbon or hydrocarbon-coated beads, optionally in a binder. Beaded polyfluorocarbon in an adhesive binder is a highly preferred hydrophobic material because it is extremely hydrophobic, in part because of the

hydrophobic nature of the polyfluorocarbon and apparently also in part due to the roughened or textured surface resulting from the beaded polyfluorocarbon.

Turning to the combined apparatus for transferring arrays of liquid samples, where the apparatus includes a microtiter-like storage plate and a transfer device that comprises an array of pins, in preferred embodiments, the faces of the pins of the transfer device are hydrophilic. Examples of materials that can be used to make hydrophilic faces for pins (or, more conveniently, whole hydrophilic pins) include plastic, metal, glass, ceramic, silicon, other crystalline materials, and biomaterials. Preferred plastics will be hydrophilic or will have their surfaces modified to improve their hydrophilicity, as for example by surface oxidation. As was the case with the hydrophilic domains described previously, a "hydrophilic" pin needs to be sufficiently hydrophilic to transfer a hydrophilic liquid. Such a pin may be hydrophobic by other standards. For example, DMSO is very hydrophilic, but still wets relatively hydrophobic materials, which would be classified as hydrophilic according to the definition used herein. An advantage of this particular apparatus compared with the prior art is that the pins can be much shorter than those that are required for reaching into the wells of conventional plates. Pin lengths can be as small as about 0.25 mm, and the range of pin lengths may be in the range of about 0.25 mm to several inches, with a range of about 0.5 to about 2.5 mm being preferred for manufacturability and tolerance considerations. Pins can also be used that are placed in a holder in which the pins can slide, so that the lengths of the pins can be adjusted during use or just before use so that the pins conform to the surface to which transfer is being made.

Finally, with respect to a method for transferring an array of liquid samples simultaneously (i.e. together as a group) from a microtiter-like storage plate to a receiving device, there are several choices of receiving devices.

The receiving device may be a microtiter plate or a microtiter-like storage plate comprising an array of wells or virtual wells that match the array of samples on the transfer device. The samples are transferred by first placing the faces of the pins of the transfer device sufficiently close to the matching wells or virtual wells that some or all of the liquid sample on the face of each pin of the transfer device is transferred to the hydrophilic domain of the well or virtual well, and second, separating the transfer device from the receiving device.

The receiving device may also be the microtiter-like storage plate disclosed herein, where the microtiter-like plate comprises an array of cavities or virtual wells and/or an array of sampling ports that match the array of samples on the transfer device. The samples are transferred by first placing the faces of the pins of the transfer device sufficiently close to the matching cavities, virtual wells, or sampling ports that some or all of the sample on the face of each pin of the transfer device is transferred to the hydrophilic domain of the cavity, virtual well, or sampling port, and second, separating the transfer device from the receiving device. Alternatively, the samples can be received in an array of virtual wells or standard cavity type plate wells by placing the face or tip of the pins in fluid held in the wells.

Finally, the receiving device may be a conventional microtiter plate which comprises an array of cavities, where the array of cavities on the receiving device matches the array of pins on the transfer device. The liquid samples on the array of pins on the transfer device are transferred by a method comprising a first step which may be one of the following three choices: (a) placing the faces of the pins of the transfer device sufficiently close to the matching cavities that some or all of the sample on the face of each pin of the transfer device is transferred to the hydrophilic domain of the cavity; (b) rinsing the samples into the cavities with a solvent; or (c) immersing the faces of the pins containing the samples into a solvent in the cavities; followed by the second step of separating the transfer device from the receiving device.

Definitions

"Close proximity" refers to the distance between the faces of the pins of the transfer device of the present invention and the sampling ports on the bottom side of a microtiter-like plate that is effective to transfer liquid samples from the sampling ports to the faces of the pins of the transfer device, or from the faces of the pins to virtual wells or the bottoms of conventional wells in another microtiter-like plate or to the surface of the destination to which the liquid sample(s) are being transferred. The optimum distance is determined by the desire to ensure that the liquid samples are transferred in uniform quantities. In general, this is accomplished by bringing the pin close enough to the sampling port to contact the droplet of liquid on the port and create a gap that is sized in such a way that the space between the face of the pin and the sampling port is filled uniformly with the liquid sample by capillary

action. The volume of liquid transferred is then determined by the surface area of the face of the pin and surface tension. In practice, the pins can generally be brought into contact with the sampling ports and then withdrawn slowly enough to allow liquid to fill the gap as the gap is created at the bottom of the sampling port Spacers can be employed between the transfer device and the microtiter plate to ensure that the optimal distance is used, but generally spacers will not be necessary.

"Spatial array" (also referred to as "array") refers to an arrangement of liquid samples in a pattern, with each liquid sample in the pattern representing an element of the spatial array. For example, liquid samples filling the wells of a 96 well microtiter plate are present in a spatial array, and the liquid sample in each well is an element of the spatial array. Liquid samples filling only some of the wells in a 96 well microtiter plate would also be present in a spatial array. In such spatial arrays, the liquid samples in each well can be the same or different from other liquid samples. The liquid samples in an array can be pure, *e.g.*, pure DMSO, pure $H_2O$, or they can be solutions of a compound or compounds, *e.g.*, a 1M NaCl solution or a DMSO-water solution. Liquid samples can contain the same or different compounds dissolved therein, and can be mixtures of liquid samples. Preferably, the liquid samples used in this invention will all have the same solvent or solvent combination to minimize variation in liquid sample size due to changes in surface tension. Spatial arrays can have any number of elements; the above-described example of a 96 well microtiter plate is meant to be illustrative only. The elements of the spatial array can be arranged in any geometric pattern. One particularly useful spatial array is formed when the liquid samples contain members of a combinatorial library.

One spatial array "matches" another spatial array if the patterns of the elements of the arrays are physically superimposable upon each other. Thus, two spatial arrays are matched if the elements are geometrically arranged in such a way that they can be superimposed. For example, the wells of two standard 96 well microtiter plates (an 8x12 area of wells having 9 mm center to center spacing) would be physically superimposable on each other, and thus the spatial arrays match.

"Hydrophilic" and "hydrophobic" have their commonly used meanings here, except that the terms are generally used in a relative sense to each other. In other words, since virtual wells rely on repulsive and attractive forces acting together, as in the case of liquids being retained in hydrophilic wells surrounded by hydrophobic domains, the hydrophilic domain just needs to be more hydrophilic than

the hydrophobic domain to achieve the desired result, and could actually be hydrophobic in common usage. Similarly, in the case of pins, "hydrophilic" pins can transfer hydrophilic liquids between the hydrophilic domains of the storage wells and sampling ports. For a liquid such as DMSO, a pin may be hydrophilic for purposes of this invention, but may be relatively hydrophobic by many other standards. Hydrophilic and hydrophobic as applied to liquids have their usual definitions.

"Microtiter-like" plates refer to plates that do not have the conventional design of wells in a substrate. Thus plates with virtual wells or with pairs of wells with connections in between are microtiter-like plates. These kinds of plates can have a higher density than conventional plates. The invention disclosed herein is for use with very high density plates (1,536 wells, with adjacent wells separated by 2.25 mm, center to center).

Virtual Wells

Virtual wells are used extensively in this invention. They are summarized in depth as follows. "Virtual wells" is a term that is used to describe wells in microtiter-like plates that hold very small volume samples on the plate and isolate them from one another by surface tension effects. In a conventional well, the samples are separated from one another by the walls of the well (see the next paragraph below). Virtual wells can be any surface modification such as protrusions or slight indentations. Generally they are slight indentations having a depth of between 0.5 nm to 500 μm, preferably about 3 nm to about 200 μm, more preferably about 10 nm to about 100 μm, and even more preferably about 10 nm to about 50 μm, as well as chemical modifications, binding sites, or other discontinuities present in slight indentations, on the plate surface that orders or retains fluid drops into a defined spatial array. Typically, the virtual wells are formed by an arrangement of relatively hydrophilic domains within relatively hydrophobic fields. Solvents that are used in biological screening and assays are generally very polar, such as DMSO or DMSO-water solutions. Solvated samples (compounds) and assay reagents are confined to the more hydrophilic domains of the virtual wells by the edges of the more hydrophobic fields.

For comparison, conventional microtiter plates contain wells that are formed by cylindrical, V, or cup-shaped indentations in the material forming the bottom plate of the microtiter plate. These wells, referred to herein as "cavities,"

generally have sidewalls and bottoms forming a depression in the bottom plate in which the samples are physically constrained under the influence of gravity. See, *e.g.*, U.S. Patent No. 5,229,161; U.S. Patent No. 4,735,778; U.S. Patent No. 4,770,856. Thus, it is the shape of the material making up conventional wells that confines the samples in those wells.

Microtiter-like plates having virtual wells lack the deep indentations found in conventional microtiter plates. The surfaces are patterned to have relatively hydrophilic domains within relatively hydrophobic fields so that a sample is physically constrained by surface tension to the more hydrophilic domains by the edges of the more hydrophobic fields. Thus, this arrangement of hydrophilic domains within hydrophobic fields creates "virtual wells." Alternatively, the "virtual wells" can result from any surface modification that physically constrains a fluid by surface tension. These virtual wells provide a location in which samples can be confined. The confined samples can be used in almost any known variety of high throughput screening assay or non-high throughput screening assay.

While the surfaces of plates containing virtual wells are generally flat to the eye or have gentle curvature, it will be understood by those skilled in the art that the hydrophilic domains may actually be extremely thin film-like areas that have been coated onto the surface of the hydrophobic fields. In some cases, the film-like areas may be a layer only a single molecule thick. In other cases, the layers may be somewhat thicker. Thus, the hydrophilic domains may actually be slightly raised compared to the hydrophobic fields. Similarly, in some embodiments, the hydrophobic fields may be extremely thin film-like areas that have been coated onto a hydrophilic surface. In no case, however, do the virtual wells consist of indentations with a depth of greater than about 1 mm, as in more conventional microtiter plates. In one preferred type of virtual well, a layer of polyfluorocarbon beads in a carrier matrix (*viz.*, TEFLON® composite material [TEFLON® beads in a matrix of supporting material]) that has been deposited onto derivatized glass. The bead and carrier layer is typically about 20 μm thick. The process described herein that can be used to produce virtual wells generally results in virtual wells that are formed by hydrophobic fields lying on hydrophilic substrates where the surface of the hydrophobic field is raised about 0.5 nm to about 500 μm, preferably about 3 nm to about 200 μm, more preferably about 10 nm to about 100 μm, and even more preferably about 10 nm to about 50 μm, relative to the surface of the hydrophilic

substrate, or where the virtual wells are hydrophilic domains lying on hydrophobic fields where the surface of the hydrophilic domains is raised about 0.5 nm to about 500 μm, preferably about 3 nm to about 200 μm, more preferably about 10 nm to about 100 μm, and even more preferably about 10 nm to about 50 μm, relative to the surface of the hydrophobic fields.

The surface of the hydrophobic fields surrounding the virtual wells need not be completely flat. In certain embodiments, is is preferred that the surface is both hydrophobic and, at least at the microscopic level, rough. It is possible that, since roughness increases the surface area of the hydrophobic field, this results in an increase in the field's apparent hydrophobicity, leading to improved performance in some instances. One method of achieving a desired degree of roughness is to make the hydrophobic field from TEFLON® beads, or other polyfluorocarbon or polyfluorocarbon-coated beads, or hydrocarbon or hydrocarbon-coated beads, in a carrier matrix. Such coatings can be obtained from commercial suppliers such as Erie Scientific (Portsmouth, NH), Cytonics, or Vellox. VELLOX® is 0.1 to 0.2 μm diameter fumed silica beads coated with a trimethyl siloxy coating in an acrylic copolymer resin layer. It is also expected that beads made from materials similar to TEFLON®, *i.e* , other polyfluorocarbons, will be suitable. Generally, when beads are used to make the hydrophobic field, the beads should have a diameter of from about 0.05 μm to about 50 μm, preferably from about 0.075 μm to about 5 μm, and even more preferably from about 0.1 μm to about 0.3 μm. Possible carrier matrices are: adhesives, waxes, epoxies, acrylics, polymers, or polyvinylidene fluoride. Another method of making such hydrophobic fields is to modify a portion of an already rough surface such as ground or sintered glass. Roughness is characterized in millionths of an inch (1 μm = 39 millionths of an inch). Typically, surfaces that are rough to about 0.1 - 1 μm or 4 to 40 millionths of an inch are most desirable.

Assays using virtual wells can be run in a microtiter-like plate having a lid or top plate as described in co-pending and commonly assigned PCT Application No. US 99/02300, filed February 3, 1999, so that evaporation is limited by the relatively tortuous path to the edge and up and over the sidewalls that the vapor would have to traverse while still allowing gas exchange for live cell assays. An incubator is used for longer incubations. Other methods of controlling evaporation are also well known in the art.

Systems Using Hydrophobic Virtual Wells

The virtual wells, sampling ports, and the like are all described in terms of hydrophilic samples being confined in hydrophilic domains surrounded by hydrophobic fields by surface tension effects. It will be readily apparent to those of skill in the arts that a mirror image kind of virtual well system could be designed using hydrophobic liquids in hydrophobic domains (the virtual well) surrounded by a hydrophilic field. Transfer devices would then require hydrophobic pins or faces of pins.

Methods of Making the Microtiter-like Storage Plates

The plates employed in the present invention can be made by a variety of techniques and from a variety of materials, some of which are explained below. Generally, there are two separate operations, which can be carried out in any sequence. First, a flat plate having an array of holes is made. Then a modification is made to the hydrophilicity of a portion of the plate so that there are regions of differing hydrophilicity, a hydrophobic region and a hydrophilic region. These will define the virtual wells. Alternatively, the hydrophilic regions and hydrophobic regions can be patterned first. Then the holes can be added to the centers of the virtual wells. It is generally more practical to make the plate with the pattern of holes first.

There is also one method that does not follow these choices. In that method, one or more very fine screens or other smooth porous materials are used to make the plate. The screen or porous support is patterned on the first side to form the storage wells and on the second side to form the sampling ports. Typically the patterning is done by silk screening a hydrophobic field onto the surface to form the wells or ports. The uncoated portions of screen then become hydrophilic domains. Alternatively, the wells and ports can be made out of different pieces of screen or porous material. After each piece is patterned one would lay one on the other such that the wells and sampling ports overlap and bind them together with an adhesive or clamp or some other kind of holding device. It is anticipated that the extreme hydrophobic nature of the silk screened coating would prevent any cross contamination between wells, even in the part of the plate between the two screens or

pieces of porous material, as it does in all of the other virtual well embodiments. This method of making the plate in two pieces has a very real advantage in that it is then necessary to silk screen only one of the sides of each piece of screen or porous material. Silk screening both sides of the screen or porous material can be difficult because the surface to be silk screened has to generally be smooth, and this precludes many methods of holding and aligning the screen or porous material while the pattern is being applied. With these methods, since the wells are made of screen or porous material, holes do not need to be drilled through the plate, since the two wells are already connected for easy transfer of liquid between the storage well and the sampling port. This eliminates or greatly simpifies one of the two operations described in the previous paragraph (obtaining a flat plate with holes).

Plates

The plate may be made from silicon, glass and many other etchable materials. In particular embodiments, the material used to make the plate is selected from the group consisting of plain glass, derivatized glass, silanized glass, glass having absorbed bio- and non-biopolymers, polystyrene and other plastics, indium tin oxide and other metal oxides, gold and other metals, and ceramics. Alumina is a preferred ceramic. Derivatized glass is glass that has had its surface chemically modified to something other than only $SiO_2$.

Silicon, alumina, glass, and other ceramic and metal plates may be purchased commercially, and there are numerous smaller companies that can be contracted to put holes in the plates and/or apply coatings. A plain unmodified plate may be modified by first etching a thin planar material such as glass, silicon, metal, or ceramic with an anisotropic etch through to a stop to get a hemispherical indentation, which will act as a well once the etch stop is removed.

Alternatively, holes may be etched through the plate if it is made from an etchable material, such as, glass, silicon, metal or ceramic. The holes may be etched using an isotropic wet etch, plasma etch, reactive ion etch, or bombardment technique. The etching can be carried out from one side, or using either an isotropic or anisotropic etch, the etching can be carried out from both sides to form an hourglass shaped hole or a linear or conical shaped hole. Alternatively, the material

may be laser drilled from one or both sides to form straight, conical or complex shaped holes. Likewise, high pressure water drilling or ultrasonic drilling may be employed to prepare the holes. Similarly, a laminating technique may be employed to stack layers of ceramic or other material with pre-cut holes to form a shaped hole. In any of the above methods, a non-noble metal may be applied and then coated with gold or platinum before a fluoropolymer is applied by silk-screening. Standard machining with a small drill bit may also be employed to prepare the holes in the wells. Alternatively, a molten or liquid material (such as spin glass, glass, or plastic) may be poured onto a form, or a softened material may be pressed into a form to make the holes. In other cases, a chemically inert, relatively hydrophilic, plastic may be employed to form the capture plate by injection molding, thermoforming, or other traditional plastic molding techniques. The methods that are currently preferred for making the plates are reactive ion etching and drilling holes in a plate made of alumina using a high powered laser.

Patterning the Domains on the Plates

A variety of techniques can be used to make the pattern of hydrophilic and hydrophobic regions to yield plates having virtual wells. In the preferred method, a polyfluorocarbon-containing substrate (e.g., TEFLON®) is silk-screened onto a glass or ceramic surface. In some instances, it may be advantageous to silk-screen the hydrophobic material onto glass or ceramic through a stiff (*e.g.*, stainless steel) mesh rather than the more typical nylon or silk mesh.

Another way to pattern the substrate is to first make a dummy pattern of the array of hydrophilic domains on a hydrophilic surface with a photoresist, then coat the entire surface with a suitably hydrophobic material, and finally selectively "lift-off" the photoresist and overlying hydrophobic material to reveal the hydrophilic pattern. This method has been carried out by using a 1:200 solution of candle wax in hexane as the hydrophobic material. After lifting off the photoresist, the wax was cleanly patterned and water repellant.

Another method is to coat a hydrophobic surface with a hydrophilic material and then make a photoresist pattern for etching the hydrophilic material away directly.

Other methods for putting patterns on the plates include just about any method where a relatively hydrophobic or relatively hydrophilic layer can be patterned on top of a contrasting, relatively hydrophilic or relatively hydrophobic layer. These methods include, but are not limited to, stamping, silk screening, or printing of a hydrophobic material on a hydrophilic surface or vice versa; layering the two surfaces and then patterning the top layer to expose the bottom layer; or directionally masking the bottom layer while adding the top layer. Some typical methods include photolithography, silk screening, plasma etching, shadow chemical vapor deposition, or using films from the proofing industry.

In particular embodiments, the hydrophilic domains are typically selected from the group consisting of: plain glass, derivatized glass, silanized glass, glass with absorbed bio- and non-biopolymers, polystyrene and other plastics, Indium Tin Oxide and other metal oxides, gold and other metals, silicon, ceramic, hydrophilic plastics, and surface-modified polystyrene. Derivatized glass is glass that has had its surface modified to be something other than $SiO_2$. The surface could also be modified with proteins, nucleic acid, or other absorbed polymers.

In particular embodiments, the hydrophobic field is selected from the group consisting of: TEFLON®, various TEFLON®-like materials (*e.g.*, polyfluorocarbons or perfluoropropene oxide) in carrier matrices such as epoxy. Other materials suitable for the hydrophobic field include waxes or oils (*e.g.* paraffin), hydrocarbons (*e.g.* polyethylene), silanizing agents (*e.g.* chlorodimethyl octyl silane), hydrophobic polymers such as polypropylene, and bifunctional materials that may bind ionically or covalently to the glass.

A preferred embodiment employs a hydrophobic field that is both hydrophobic and, at least at the microscopic level, rough. One method of achieving a desired degree of roughness is to make the hydrophobic field from polyfluorocarbon or polyfluorocarbon-coated beads, or hydrocarbon or hydrocarbon-coated beads, in a carrier matrix. The coatings for such beads can be obtained from commercial suppliers such as Erie Scientific (Portsmouth, NH) or Cytonics or Vellox. VELLOX® coating is 0.1 to 0.2 μm diameter fumed silica beads coated with a trimethylsiloxy coating in an acrylic copolymer resin layer. The polyfluorocarbon beads are typically 0.1 – 5 microns in diameter.

In particularly preferred embodiments of the above-described novel plate, the plate is alumina, the hydrophilic domains are alumina, which may be

derivatized, and the hydrophobic field is a silk screened polyfluorocarbon bead based coating .

Methods of Making the Transfer Device

The transfer device can be made by numerous methods. Three of these are described in detail in Examples 5, 6 and 7, and are preferred methods. These preferred methods are:

milling of plastics using a CNC (computer numerical control) machine,

removing metal from a plate by the method of Electrical Discharge Machining (EDM), which can be used only on metals and other conductive materials,

and insertion of the desired pre-formed pins into a pc board (FR4 epoxy resin) or other carrier such as aluminum or other metal, plastic sheet, a molded piece, or other easily machined or formed planar substrate. In this last method, the pins can be inserted with a tight (non-slip) fit or a slip fit, where a slip fit is tight enough to align the pins but permits slippage of the pins when a force is applied, so that the tips of the pins can all be lined up in the same plane. When a slip fit is used, the pin is generally held in the pc board or other carrier by a head or other enlargement at one end of the pin so that the pin does not fall out of the pc board or other carrier.

Other methods include:

cold forging of a relatively soft metal with a low melting point. In this method, the metal that is being cold forged is subjected to a sudden intense pressure when a very heavy mold is smashed into it;

injection molding, which would generally be used for thermoplastics;

casting molten material into an open mold;

micromachining into the surface of a material selected from the group consisting of glass, silicon and other crystalline materials by a process selected from the group consisting of anisotropic, isotropic, plasma, and reactive ion etching;

laser cutting into the surface of a material selected from the group consisting of glass, silicon, or other crystalline material; metal or other conductive materials; and plastic;

molding from a material selected from the group consisting of plastic, glass, and metal by conventional macroscopic methods or standard microfabrication methods used in micromachining, such as LIGA (x-ray lithography, electrodisposition, and molding).

The following non-limiting examples are presented to better illustrate the invention.

## EXAMPLE 1

A silicon wafer (six inch diameter x about 500 microns thick) is made into a substrate for use in making microtiter-like storage plates by forming a high density 48 x 32 array of holes (1,536 holes). Reactive ion etching (R.I.E.) was used to make the holes. The R.I. E. was done by Kionix, Ithaca, NY. The holes have a diameter of about 200 microns, with a 2.25 mm spacing between holes.

The wafer was then made into a microtiter-like storage plate by applying coatings of polyfluorocarbon beads in a binder to both sides of the plate by hand method. The coating was cured by heating the coated silicon substrate to 150°C for 30 minutes. The features needed to create the hydrophilic domains and hydrophobic fields were obtained by applying masks to the silk screens before the coating was applied. The mask was a photopolymer of the type that is used in making photoresists in the electronics industry. The uncoated areas around the storage wells were circles having a diameter of about 1.5 mm, and the uncoated areas around the sampling ports were circles having a diameter of about 600 microns. The silicon substrate became the hydrophilic domains in the finished product.

## EXAMPLE 2

A full-size (12.0 cm x 7.80 cm) ceramic plate was laser drilled to yield a high density plate having 1536 holes.The plate was 0.50 mm thick with a flatness profile of 50 microns over the surface of the plate. The plate was made of alumina (Catalog No. AD-96 from Coors Ceramics Co., 1100 Commerce Park Drive, Oak Ridge, Tenessee 37830). The holes on the second (bottom) side of the

plate were made with a high intensity carbon dioxide laser, and the holes on the first (top) side of the plate are made with a high powered excimer laser. This yielded small cup-shaped openings having a diameter of about 220 to 240 microns on both the top and the bottom of the plate. The holes that pass through the plate between the cup-shaped openings are single channels having a diameter of about 85-275 microns. Alternatively, at the discretion of the person making the plate, the connections between the wells on the top and bottom of the plate can comprise groups of several holes (e.g. 3-4), which can also be made by laser drilling. Further testing shows that only a carbon dioxide laser is needed to make the holes in the alumina and that the holes can be considerably larger.

The plate was then made into a microtiter-like storage plate by applying coatings of polyfluorocarbon beads in a binder to both sides of the plate by a silk screening method. The coating was cured by heating the coated silicon substrate to 150°C for 30 minutes. The silk screening was performed by Erie Scientific, Portsmouth, NH. The features needed to create the hydrophilic domains and hydrophobic fields were obtained by applying masks to the silk screens before the coating was applied. The mask was a photopolymer of the type that is used in making photoresists in the electronics industry. The uncoated areas around the storage wells were circles having a diameter of about 1.5 mm, and the uncoated areas around the sampling ports were circles having a diameter of about 600 microns. The silicon substrate became the hydrophilic domains in the finished product.

## EXAMPLE 3

An alumina plate (12 cm x 7.8 cm x 3 mm) was made by Westar Automation, Inc., Fairless Hills, PA, by stacking multiple layers of thin plates made from alumina particles and a binder (pre-ceramic "greenbodies') and then sintering the stacked plates into a ceramic plate , while at the same time burning away the binder. The greenbodies had the needed array of holes formed into them prior to sintering. The holes in the top unsintered plate were larger than the holes in the other plates so that the top of each hole on the side of the plate with the storage wells would be larger than the corresponding sampling port on the other side of the plate. It is now believed

that a hole having the same diameter all the way through would work just as well. The finished alumina plate after sintering had a 48 x 32 array of 1536 holes spaced 2.25 mm apart. The holes went all the way through the alumina plate and had a 350 micron opening on one side, with the remainder of each hole having a diameter of about 175 microns.

The alumina plate was made into a microtiter-like plate as described in Example 2 by applying a coating of fluorocarbon beads to both sides through a masked silk screen to yield a plate with the desired features.

## EXAMPLE 4

A full-size ceramic plate having the dimensions, hole spacings, and hole sizes of Example 1 is made by molding a hydrogel pre-ceramic silica plate, including holes, followed by high temperature sintering.

## EXAMPLE 5

A transfer device is milled out of a piece of plastic (either acrylic or polycarbonate). The milling process is carried out in a CNC machine (Computer Numerical Control machine) and involves mechanical removal of the plastic using a precisely controlled rotary bit to achieve the fine features needed for this device. Plates having dimensions of 120 x 78 mm and a thickness of either 6.35 mm or 12.7 mm were milled into 1536 pin arrays (32 x 48 pins) having spacings of 2.25 mm (center to center). The pins were square and had square faces on the tips, having a length on one side of 300, 350, and 500 microns in various trials. The length of the pins was 0.5 mm. Prototype 5x5 arrays of pins having the same shapes and dimensions were successfully used to transfer liquids from the sampling ports of a plate to other glass plates.

## EXAMPLE 6

Stainless steel and Monel plates having dimensions of 120 x 78 mm were made into transfer devices having pin arrays using (wire) Electrical Discharge

Machining (EDM) as the method of removing metal to get the desired product. In this case, wire EDM was the precise method that was used. A full size 1536 pin array was made with 2.25 mm spacings. The pins had square cross sections and square faces. The lengths of the sides of the square faces were 400 mm. The heights of the pins were 3 mm.

These transfer devices were successfully used to make transfers from the sampling ports of the plates taught herein to other plates.

## EXAMPLE 7

A 78mm x 120 mm epoxy resin plate is predrilled with 1536, 1280 or 256 holes in an array such that the holes can accommodate the insertion of gold plated pins to form a transfer device which has an array of pins that matches the array of wells and sampling ports in a storage plate. The pins are fitted into the holes with a slip fit, permitting the pins to adjust their lengths to the surface defined by the wells or sampling ports during or before use. The heads of the pins prevent the pins from slipping through the holes in the plate. The assembly is backed with one or more plates that prevents the pins from falling out of the plate when the plate is inverted. The entire assembly is typically glued into a frame for use.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED:

1. A microtiter-like storage plate, said plate having a first side and a second side, comprising:

a) an array of storage wells on the first side of said plate; and

b) an array of sampling ports on the second side of said plate,

wherein the storage wells on the first side of the plate and the sampling ports on the second side of said plate are paired, with each storage well that is on the first side of the plate and each sampling port that is on the second side of said plate that are paired being opposite each other on the two sides of the plate to form a pair of one storage well and one sampling port, with each pair of one storage well and one sampling port being connected through the plate such that liquid can flow between the paired storage well and sampling port, but not laterally in the plane of the plate to other storage wells or sampling ports;

wherein the sampling ports on the second side of the plate are virtual wells, each virtual well comprising a hydrophilic domain surrounded by a hydrophobic field;

wherein the storage wells on the first side of the plate have the capacity to hold a larger volume of liquid than the sampling ports on the second side of the plate, and the sampling ports on the second side of the plate hold a volume of liquid that varies little with changes in the combined volume of liquid that may be in the paired storage wells and sampling ports on the two sides of the plate.

2. A microtiter-like storage plate as recited in Claim 1, wherein the storage wells on the first side of the plate are selected from the group consisting of cavities and virtual wells, each of said virtual wells comprising a hydrophilic domain surrounded by a hydrophobic field.

3. A microtiter-like storage plate as recited in Claim 2, wherein the storage wells on the first side of the plate are cavities.

4. A microtiter-like storage plate as recited in Claim 3, wherein the storage wells on the first side of the plate have a volume in the range of about 0.001 ml to about 1ml.

5. A microtiter-like storage plate as recited in Claim 2, wherein the storage wells on the first side of the plate are virtual wells, each virtual well comprising a hydrophilic domain surrounded by a hydrophobic field, said hydrophilic domain having an area of about 0.38 $mm^2$ to about 19 $mm^2$, and wherein the hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.002 $mm^2$ to about 6.75 $mm^2$, with the proviso that the areas of the hydrophilic domains of the sampling ports are smaller than the areas of the hydrophilic domains of the storage wells.

6. A microtiter-like storage plate as recited in Claim 2, wherein the storage wells on the first side of the plate are virtual wells, each virtual well comprising a hydrophilic domain surrounded by a hydrophobic field, said hydrophilic domain having an area of about 0.75 $mm^2$ to about 3.1 $mm^2$, and wherein the hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.03 $mm^2$ to about 0.75 $mm^2$, with the proviso that the areas of the hydrophilic domains of the sampling ports are smaller than the areas of the hydrophilic domains of the storage wells.

7. A microtiter-like storage plate as recited in Claim 6, wherein the hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.2 $mm^2$ to about 0.5 $mm^2$.

8. A microtiter-like storage plate as recited in Claim 3, wherein the hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.002 $mm^2$ to about 6.75 $mm^2$.

9. A microtiter-like storage plate as recited in Claim 3, wherein the hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.03 $mm^2$ to about 0.75 $mm^2$.

10. A microtiter-like storage plate as recited in Claim 3, wherein the hydrophilic domains of the sampling ports on the second side of the plate have an area in the range of about 0.2 $mm^2$ to about 0.5 $mm^2$.

11. A microtiter-like storage plate as recited in Claim 1, wherein each storage well on the first side and each sampling port on the second side of said plate that are paired together are connected by a channel.

12. A microtiter-like storage plate as recited in Claim 11, wherein said channel may be approximately cylindrical in shape, or said channel may be flared at the openings on the first and/or second sides of the plate; said channel having a cross-sectional area measured perpendicular to the walls of the channel in the range of about $1x10^{-6}$ to about 1.0 $mm^2$ at the narrowest part of the channel between the storage well and the sampling port.

13. A microtiter-like storage plate as recited in Claim 1, wherein each storage well on the first side and each sampling port on the second side of said plate that are paired together are connected by a number of channels or perforations, or by a porous or woven support that is hydrophilic relative to the hydrophobic field surrounding the hydrophilic domains of the virtual wells.

14. The microtiter-like storage plate as recited in Claim 13, wherein the pairs of storage wells and sampling ports are connected by a porous or woven support that is hydrophilic relative to the hydrophobic field surrounding the hydrophilic domains of the virtual wells, wherein the porous or woven support is selected from woven or fritted ceramic, silicon, glass, metal, hydrophilic plastic, a plastic with a hydrophilic surface, sponge, and zeolites.

15. The microtiter-like storage plate as recited in Claim 2, wherein the hydrophilic domains of the virtual wells on the first side of the plate and of the sampling ports on the second side of the plate are each made of a material selected from the group consisting of plain glass, derivatized glass, silanized glass, glass with absorbed biopolymer or non-biopolymer, indium tin oxide, other metal oxides, gold

or other metals, silicon, ceramic, hydrophilic plastics, and surface-modified polystyrene.

16. The microtiter-like storage plate as recited in Claim 2, wherein the hydrophobic field is selected from the group consisting of:

polyfluorocarbons or polyfluorocarbon beads; TEFLON® or TEFLON® beads; perfluoropropene polymer; paraffin or other waxes or oils; polyethylene or other hydrocarbons; glass treated with chlorodimethyl octyl silane or other silanizing agents; polypropylene or other hydrophobic polymers; bicomponent materials containing beads or other insoluble hydrophobic materials, optionally in a binder; polyfluorocarbon beads or polyfluorocarbon-coated beads, optionally in a binder; and hydrocarbon or hydrocarbon-coated beads, optionally in a binder.

17. The microtiter-like storage plate as recited in Claim 16, wherein the hydrophobic field is beaded polyfluorocarbon in an adhesive binder.

18. A combined apparatus for transferring an array of liquid samples of approximately constant volume from a microtiter-like plate, comprising

(1) the microtiter-like storage plate of Claim 1, and

(2) a transfer device, said transfer device comprising an array of pins;

wherein said array matches the array of sampling ports on the second side of the plate;
wherein each pin has a face at one end; and
wherein said faces are all in a single plane.

19. The combined apparatus as recited in Claim 18, wherein the pins are moveable, and the faces of the pins are all in a single plane during use.

20. The combined apparatus as recited in Claim 18, wherein the faces of the pins of said transfer device are hydrophilic.

21. The combined apparatus as recited in Claim 20, wherein the pins are made from a material selected from the group consisting of plastic, metal, glass, ceramic, silicon, other crystalline materials, and biomaterials.

22. The combined apparatus as recited in Claim 18, wherein the length of the pins is in the range of about 0.25 mm to about 2.5 mm.

23. A method for transferring an array of liquid samples simultaneously from a microtiter-like plate to a receiving device comprising the steps of:

(1) providing the combined apparatus recited in Claim 18, wherein said microtiter-like storage plate also comprises an array of liquid samples;

(2) placing said transfer device in close proximity to said microtiter-like storage plate, so that the faces of the pins of said transfer device and the matching sampling ports of said microtiter-like storage plate are sufficiently close that liquid from the sampling ports is transferred to the faces of the pins;

(3) separating the transfer device and the microtiter-like plate; and

(4) transferring the array of samples from the transfer device to a receiving device.

24. The method as recited in Claim 23, wherein said receiving device is a microtiter plate or a microtiter-like plate comprising an array of wells or virtual wells that match the array of samples on said transfer device, and said samples are transferred by first placing the faces of the pins of said transfer device sufficiently close to the matching wells or virtual wells that some or all of the sample on the face of each pin of said transfer device is transferred to the hydrophilic domain of the well or virtual well, and second, separating the transfer device from the receiving device.

25. The method as recited in Claim 23, wherein said receiving device is the microtiter-like storage plate recited in Claim 1, said microtiter-like storage plate comprising an array of cavities or virtual wells and/or an array of sampling ports that match the array of samples on said transfer device, and said samples are transferred by first placing the faces of the pins of said transfer device sufficiently close to the matching cavities, virtual wells, or sampling ports that some or all of the sample on the face of each pin of said transfer device is transferred to the hydrophilic domain of the cavity, virtual well, or sampling port, and second, separating the transfer device from the receiving device.

26. The method as recited in Claim 23, wherein said receiving device in step (4) is a microtiter plate which comprises an array of cavities, wherein said array of cavities on the receiving device matches the array of pins on the transfer device, and the liquid samples on the array of pins on said transfer device are transferred by a method comprising a first step selected from the group consisting of (a) placing the faces of the pins of said transfer device sufficiently close to the matching cavities that some or all of the sample on the face of each pin of said transfer device is transferred to the hydrophilic domain of the cavity; (b) rinsing the samples into the cavities with a solvent; or (c) by immersing the faces of the pins containing the samples into a solvent in the cavities; followed by the second step of separating the transfer device from the receiving device.

27. A microtiter-like storage plate as recited in Claim 1, wherein the storage wells on the first side of the plate are virtual wells comprising a hydrophilic domain surrounded by a hydrophobic field, wherein the plate comprises at least two layers of porous material and/or fine screen, the porous layer and/or fine screen being a hydrophilic material, wherein one layer of screen or porous material comprises a hydrophobic coating applied to one or both sides but with uncoated openings for the the hydrophilic portion of the virtual storage wells, and wherein one layer of screen or porous material comprises a hydrophobic coating applied to one or both sides but with uncoated openings for the hydrophilic domains of the sampling ports.

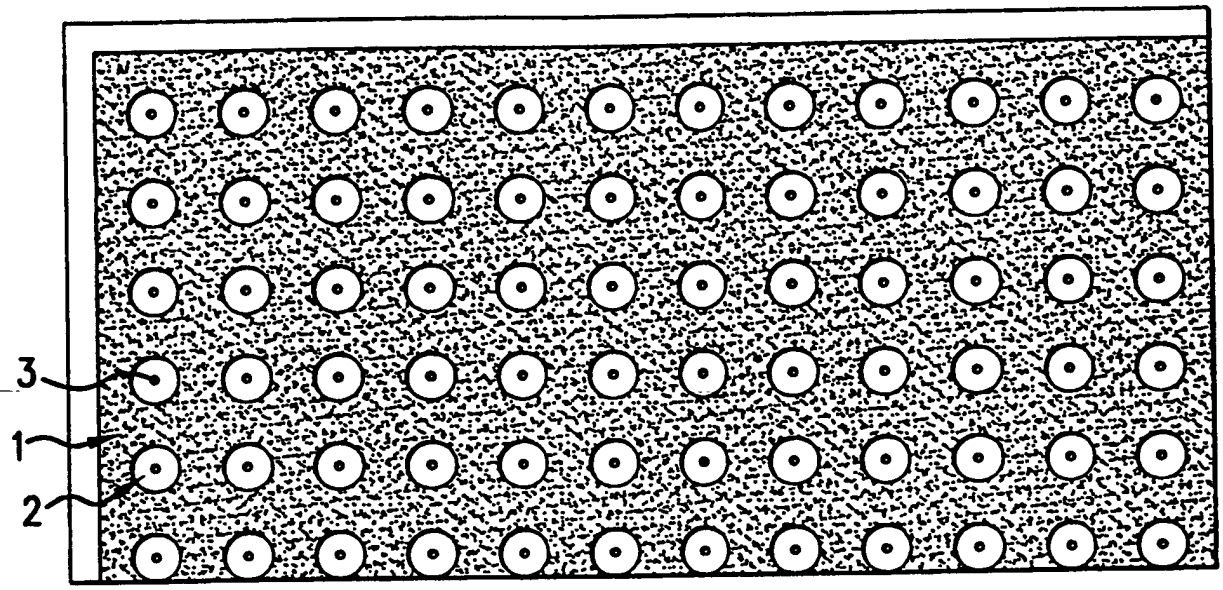


FIG. 1A

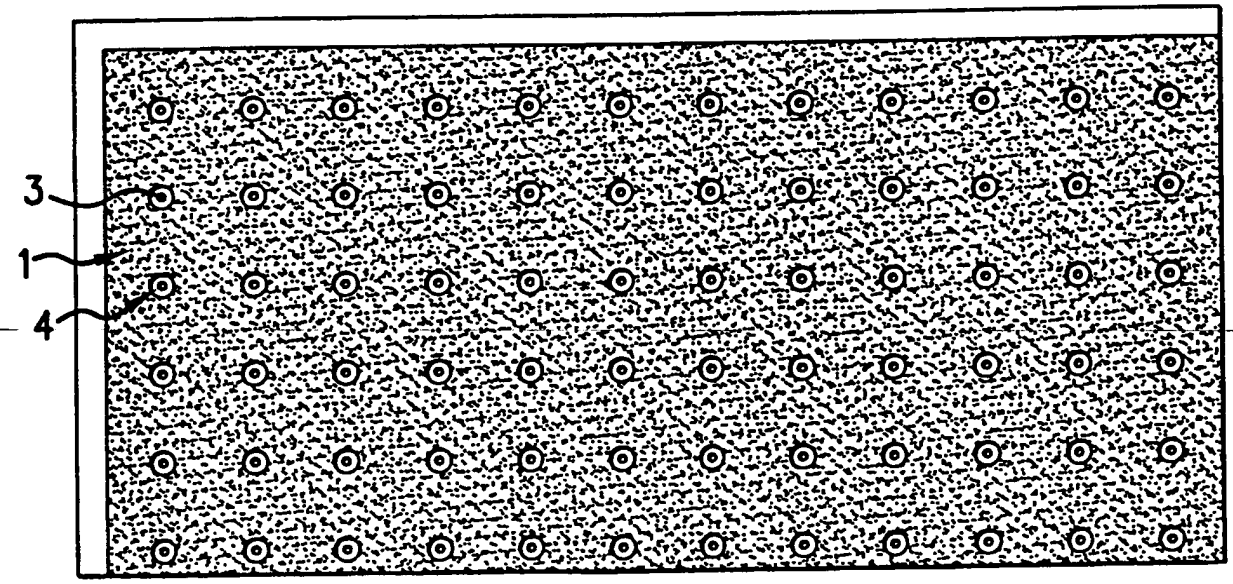


FIG 1B

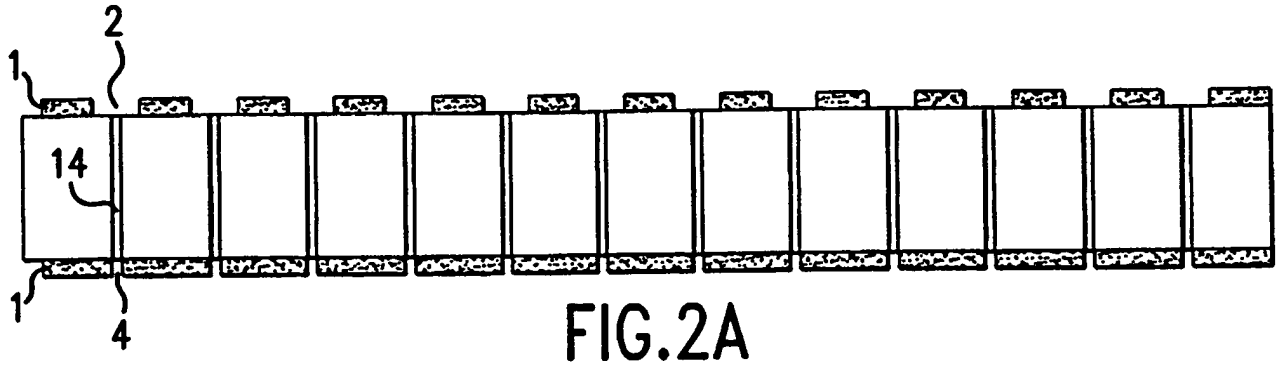


FIG.2A

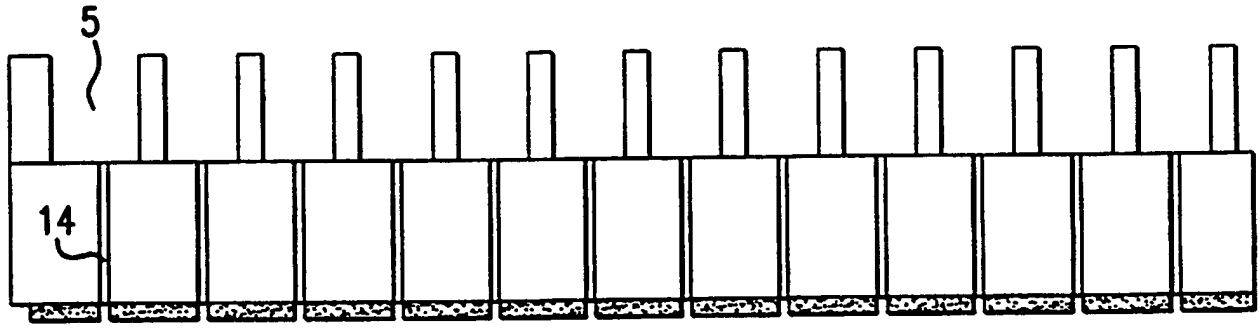


FIG.2B

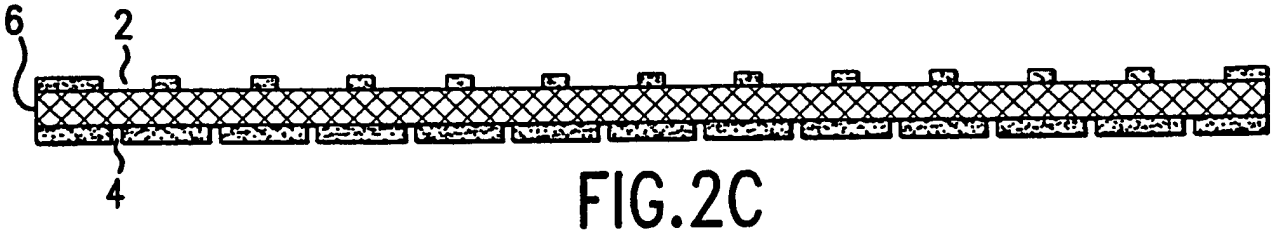


FIG.2C

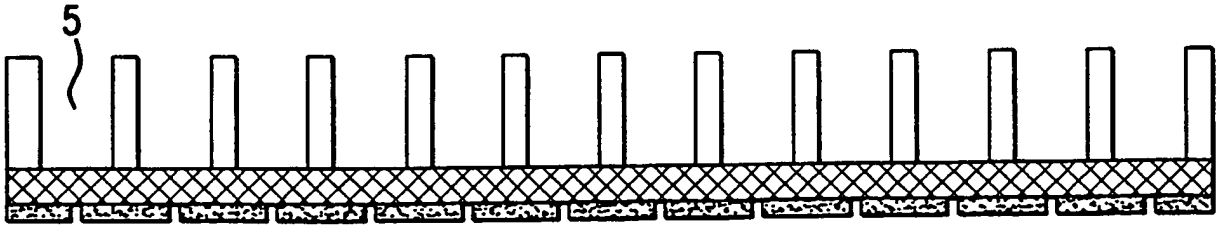


FIG.2D

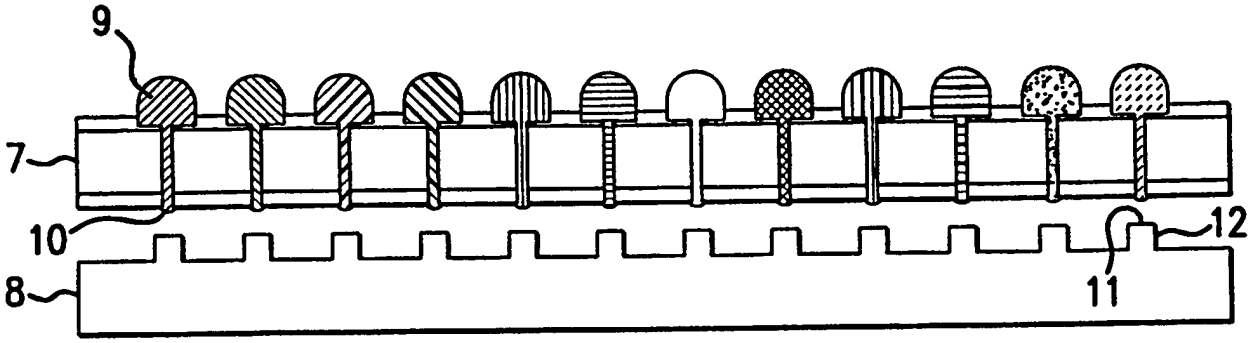


FIG.3A

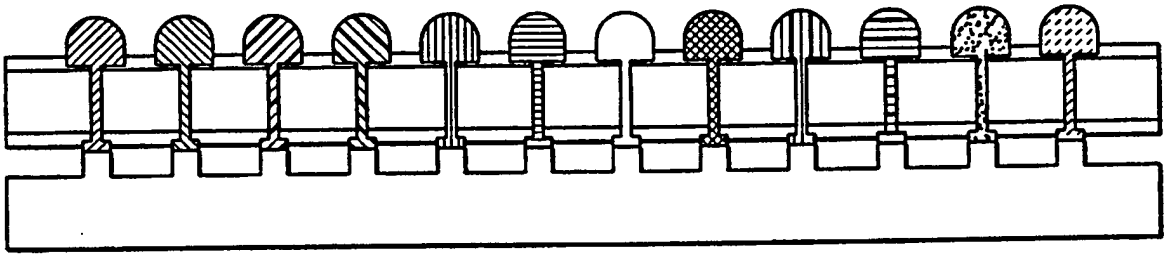

FIG.3B

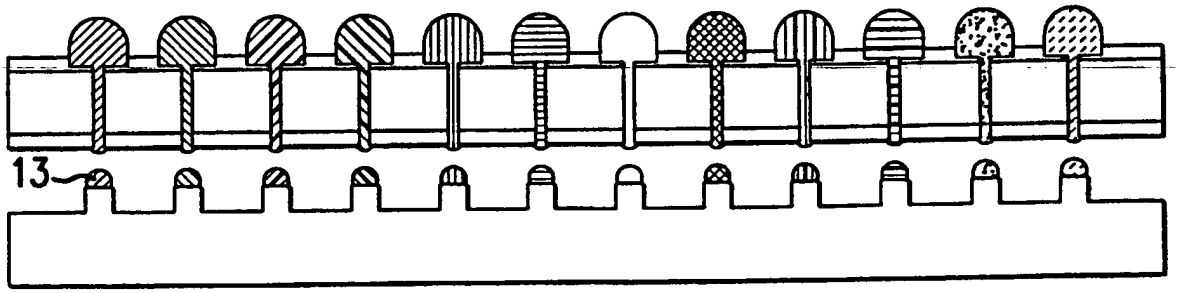


FIG.3C

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19968

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :B01L 3/02; G01N 1/10
US CL :422/100, 99; 436/180

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/99, 100, 103; 436/174, 179, 180; 73/863, 863.31, 863.32, 864.91; 435/293, 300

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST search on: microwell, microtiter, microplate, virtual well, hydrophobic, hydrophilic, liquid or fluid dispens$4

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 3,736,042 A (MARKOVITS et al) 29 May 1973, entire document. | NONEN |
| A | US 4,011,350 A (MARKOVITS et al) 08 March 1977, entire document. | |
| A | US 4,231,660 A (REMY et al) 04 November 1980, entire document. | |
| A | US 4,705,705 A (BROSS) 10 November 1987, entire document. | |
| A | US 4,798,706 A (BRIGATI) 17 January 1989, entire document. | |
| A | US 5,985,551 A (BRENNAN) 16 November 1999, entire document. | |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 25 SEPTEMBER 2000 | 18 OCT 2000 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231 | Authorized officer<br>DWAYNE K. HANDY |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19968

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 99/39829 A (GARYANTES) 12 August 1999, entire document. | |